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Simplex Centroid Mixture Design for Optimizing and Promoting the Anaerobic Co-Digestion Performance of Sheep Blood and Cheese Whey

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ABSTRACT

Reduced emissions of greenhouse gases and global warming can be made possible by discovering alternative energies and reduced dependence on fossil fuels. Biogas is considered as one of the alternatives to fossil fuels. This study investigates anaerobic co-digestion for the development of biogas with sheep blood and cheese whey. Digested cow manure was used as inoculum. Using the Design Expert 10 program and within the context of mixture design, the experiments were designed. Then, 22 experimental digesters with a volume of 500 mL were considered for doing the experiments considering the design output provided by the software. Each one was filled with 300 mL of different compositions of three matters. The digesters were kept in the mesophilic temperature range (37 °C) for 21 days. Biogas was measured using the BMP test on a daily basis. According to the experimental findings, the best composition included 35 % sheep blood, 35 % cheese whey, and 30 % inoculum. This biogas composition produced a biogas yield of 146.66 mL/g vs. The amount of methane production in this compound was 73.33 mL/g vs. After modeling, the Design Expert software predicted an optimal composition including 44 % sheep blood, 24 % cheese whey, and 32 % inoculum. Biogas yield of this prediction was 143 mL/g vs. The findings show that in order to overcome acidification in digestion of matters such as cheese whey, a composition of matters with higher pH stability can be used to increase the amount of biogas and methane produced in a particular period. Furthermore, using inoculum accelerates the digestion operations due to existence of many microorganisms and saves time and energy.

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1. INTRODUCTION

Over the last few years, renewable energy resources received much attention [1]. Jungles, agricultural resources, urban and industrial organic sewage, urban solid waste, poultry manure, and livestock and biogas are among the major classifications for use. From a socio-economic perspective, biogas not only reduces the costs associated with destroying the wastes, but also has very low raw materials cost. Furthermore, the price of biogas is lower than diesel and gasoline. Generally, it refers to the gas obtained from anaerobic digestion units, a promising method for satisfying global energy needs and provides multiple environmental advantages [2].

Anaerobic digestion is an efficient and appropriate technology for managing organic matters. It involves several organisms with final effective environmental conditions. The type and composition of the substrate are effective in biogas production performance. Organic matters are mainly a mixture of proteins, fats, and carbohydrates that can be broken down into simpler compositions using microorganisms in an anaerobic environment during the following processes: hydrolysis, acidification, acetate production, and methanogenesis. The process of biogas production from different organic matters is mostly dependent on the substrate content, while the chemical compositions and their biodegradation are key factors in biogas and methane production [3].

Anaerobic co-digestion is anaerobic digestion of two or more substrates and is a promising method for overcoming the shortcomings of mono-digestion (involving one substrate) and improving economic satisfaction with anaerobic digestion units due to more methane production. Besides producing more biogas, this method has other advantages such as improved process stability, balance of nutrients, greater moisture in the digester feed, reduced greenhouse gases, contributory effects of microorganisms, increased load of biodegradable organic matter, and cost. Concurrent anaerobic digestion, compared to mono-digestion, will increase biogas yield from 25 to 400 percent [4].

Effective factors in anaerobic digestion include temperature regime, C / N proportion, pH, Organic Loading Rate (OLR), and Solid Retention Time (SRT) [5]. Anaerobic co-digestion of slaughterhouse waste has been considered as an applicable proposal to increase biogas production in the traditional digesters. Slaughterhouse wastes are characterized by high nitrogen contents. This effect adds an appropriate substrate for accomplishment of a composition with a balanced C/N ratio, which increases nitrogen concentration and biogas yield [6, 7]. Although nitrogen for anaerobic microorganisms is a

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necessary nutrient, ammoniac concentration has been reported in digestion of wastes with high nitrogen content [8]. Nevertheless, recently, different mixtures of slaughterhouse waste and blood have been studied due to their high biochemical methane potential. Accordingly, Alvarez and Liden [9] examined digestion of a mixture of the contents of cow gut and that of pig and blood along with fruits and vegetable waste and fertilizer.

In addition to high Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD), the cheese manufacturing industry produces a large volume of waste water with high durability [10]. Cheese whey is one of the peripheral products of cheese with a considerable amount of lactose (45-50 g/L), protein (6-8 g/L), carbohydrates (4-5 %), fat (4-5 g/L), and mineral salts (8-10 % of the dry extract). Cheese whey also includes a considerable amount of lactic acid, citric acid, and Vitamin Bs. Therefore, this substrate has the capacity for biological changes. High COD and the tendency toward quick acidification is very difficult [10].

Elena Cominoa [11] examined the biogas performance of the mixture of cow manure and cheese whey, methane yield, and the efficiency of chemical and biological oxygen demand removal at 35 °C. Production of sustainable biogas from volatile solids was 621 liters per kilogram within 42 days in a mixture containing 50 % fertilizer and 50 % cheese whey. Methane concentration in biogas was 55 %. Maximum Chemical Oxygen Demand (COD) removal rates were 82 % and 90 %, respectively. Rico et al. (2015) conducted another study with the purpose of creating a combinational process involving superioritys of co-digestion of cheese whey and fertilizer and short hydraulic retention time with high load. This process of co-digestion happened in an Up-flow anaerobic sludge blanket (UASB) reactor. In the constant hydraulic retention time of 2.2 days and by increasing the ratio of cheese whey in the feed, the system displayed stable operation up to a 75 % cheese whey deduction in the feed, with a practical organic loading rate of 19.4 kg COD m^{-3} d^{-1}, obtaining a 94.7 % COD removal and a methane production rate of 6.4 m^3 CH_4 m^{-3} d^{-1}.

Considering the fact that few studies have been conducted on digestion of blood waste for biogas production, the present study examined biogas production by co-digestion of sheep's blood and cheese whey and inoculum (cow manure) for the first time. The purpose of the present study was to achieve an optimal composition of inoculum (cow manure), sheep's blood, and cheese whey to maximize biogas and methane production. Therefore, the independent variable in the present study was different compositions of substrate and the dependent variables included biogas yield and the methane existing in it. The studies were developed using the software Design Expert and in the framework of mixture design. Co-digestion of these two matters in different proportions was analyzed and the best proportion in a mixture for maximizing production was identified.

2. EXPERIMENTAL

2.1. Preparing the substrate materials and inoculum for co-digestion

Inoculum was prepared from fresh cow manure. This manure was kept at a hydraulic retention time of 90 days in a separate reactor [12] and was then used in the experiments. Sheep blood was obtained from industrial slaughterhouse of Salehabad in Hamedan and then, was pasteurized at 70 °C for 60 minutes and used in digestion experiments [6]. cheese whey was obtained from a local dairy production workshop and used in the experiments instantly.

2.2. Analytical methods used for determining the substrate features

2.2.1. Total solids

Measuring the level of solids was based on a standard method [13]. On this basis, the matters used in the experiments were kept in an oven for 24 hours at 105 °C. According to Equation 1 TS value was measured.

$$TS = (M_1 \times 100)/M_0 \quad (1)$$

where TS represents total solids, M_0 is the initial weight before drying, and M_1 denotes the final weight of the matter after drying.

2.2.2. Volatile solids

The suspended solids were measured according to the standard method [13] based on the lost weight of the dry matter that included the same samples used in an oven for measuring TS. Accordingly, the matter extracted from the oven was put in a furnace at 550 °C for six hours and VS was measured through Equation 2.

$$VS = (M_1 - M_2) \times 100/M_1 \quad (2)$$

where VS represents the amount of suspended solids, M_1 shows the weight of the dry matter, and M_2 is the final weight after drying at 550 °C [14].

2.2.3. pH

pH was measured using a pH meter model PH-230SD made in Taiwan. The obtained properties are presented in Table 1.

Table 1. Substrate properties used in the experiment

Property	Inoculum	Sheep blood	Cheese whey
Total solids (TS %)	13.7	10.7	7.4
Volatile solids (VS %)	89.9	94.2	90.5
pH	7	6.8	5.4

2.3. Experimental setup

2.3.1. Biochemical methane potential test and biogas measurement

The potential test for biochemical methane is an anaerobic batch digestion process that is typically used to determine the quantity of biogas and methane derived from organic substrates.Two commonly used BMP test methods include volumetric and monomeric [15]. In the present study, the volumetric method was used. The substrate mixture was prepared according to Table 2 and put in 500-milliliter blue-capped glasses. From each mixture, 300 mL was put in each glass. The glasses were kept in water bath and at 37 °C for 21 days and the gas yield of the reactors was measured.

Each bottle had two taps, one as the produced gas outlet and one for sampling from the substrate. The gas coming out of the bottle entered the gas collector bottle. This bottle contained water and water came out of it as much as the gas inflow. pH of the reactors was measured once a day and if reduced, using 4 normal caustic soda (NaOH), it was brought back to the normal range (6.5-8.2).

2.3.2. Measuring methane percentage

In the anaerobic digestion process, most of the gases emitted include methane (CH_4) and CO_2. Methane content was measured using an apparatus called Einhorn. It is first filled with liquid sodium hydroxide (NaOH) and then, 5 mL of biogas was injected into it. The injected biogas passed through the sodium hydroxide content inside the apparatus and its CO_2 was absorbed. The remaining gas at the top of the apparatus shows the amount of methane in 5 mL of biogas [16].

2.4. Design of experiments (DOE)

Different percentages of the mixture were determined by the Design Expert software. For this purpose, the mixture design was used. The design of the mixture is a surface response design that allows the effect of changing the ratios between variables to be investigated. The domain is an ordered figure in this design with as many vertices as components, in a space of dimensionality equal to the number of components minus one. An equilateral triangle whose vertices correspond to combinations containing 100 percent of a single component is a mixture design of three components. A mixture that does not have one of the three components represents the sides of a triangle (Figure 1). Simplex designs are used to measure the effects of mixture components on the response variable, and simplex lattice or simplex centroid configuration may be chosen among them. The models used in the design of the mixture are different from the polynomials used for independent variables on the response surface. The well-known Scheffe polynominals that can be linear, quadratic, full-cubic and special cubic models are these models [17].

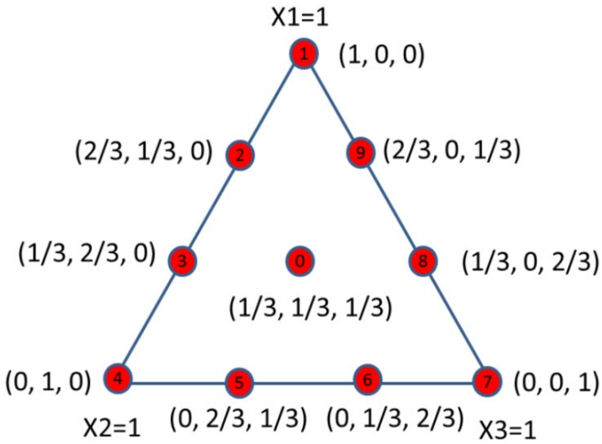

Figure 1. Augmented simplex centroid design plan used for experiments

Based on this design and according to the software output with a central point, four additional model points, five lack-of-fit points and three repetition points, 22 different compositions of substrate matters were loaded, as presented in Table 2. The experiment was carried out for 21 days. This retention time was obtained from pre-experiments.

To assess the effect of co-digestion on the output of biogas as a response variable, three components were used, which represented the percentage of each matter in the digester mixture. The amount of these components is 0-0.8 for sheep blood and cheese whey and 0.2-0.6 for inoculum. Accordingly, if "q" denotes the number of matters composing the system under study and xi represents the component of the mixture, the mixture in each reactor will be equal to:

$$\sum_{i=1}^{q} x_i = x_1 + x_2 + \ldots + x_q = 1.0 \quad x_i > 0 \quad i = 1, 2, 3, \ldots, q$$

Table 2. The order of different substrate mixtures based on the output from Design Expert software and responses

Run	Build type	Space type	Component 1	Component 2	Component 3	Response 1	Response 2
			A: blood	B: cheesse whey	C: inoculum	Biogas	CH_4
			%	%	%	mL/g vs	mL/g vs
1	Replicate	Vertex	80	0	20	122.92	38.11
2	Model	AxialCB	55	15	30	141.69	70.84
3	Lack of fit	Interior	35	35	30	146.66	73.33
4	Model	CentEdge	20	20	60	96.54	59.21
5	Model	ThirdEdge	0	66.6667	33.3333	111.51	65.04
6	Lack of fit	CentEdge	40	40	20	105.19	43.83
7	Lack of fit	Center	30	30	40	135.72	67.41
8	Lack of fit	TripBlend	40	13.3333	46.6667	113.00	47.83
9	Model	ThirdEdge	26.6667	53.3333	20	127.60	36.58
10	Model	Vertex	0	40	60	98.39	77.40
11	Model	Vertex	40	0	60	86.07	38.45
12	Model	Vertex	0	80	20	102.21	58.94
13	Lack of fit	TripBlend	13.3333	40	46.6667	112.18	49.36
14	Model	ThirdEdge	53.3333	26.6667	20	119.66	39.09
15	Model	ThirdEdge	0	53.3333	46.6667	108.88	71.86
16	Model	Interior	25	25	50	110.09	70.82
17	Replicate	Vertex	0	40	60	95.81	76.01
18	Model	ThirdEdge	53.3333	0	46.6667	95.75	54.89
19	Replicate	Vertex	0	80	20	110.73	57.94
20	Model	ThirdEdge	66.6667	0	33.3333	108.45	39.77
21	Model	AxialCB	15	55	30	110.68	44.64
22	Model	Vertex	80	0	20	118.24	31.92

3. RESULTS AND DISCUSSION

3.1. Cumulative biogas production analysis

Cumulative charts related to biogas production in different reactors are presented in Figures 2. According to Dareioti and Kornaros [18], care should be taken to ensure that alkalinity is high enough to prevent system instability as a result of likely accumulation of volatile fatty acids. Therefore, it can be concluded that Reactors 5, 10, 12, 17, and 19 had a biogas yield of less than 115 mL/g vs due to the lack of blood. These reactors, which had at least 40 % of cheese whey, faced a severe pH decline leading to reduced biogas production. Reactor 6 is of the same class, but had 40 % of blood in its mixture. However, due to the high cheese whey content (40 %), it had a similar performance to the previous reactors. Furthermore, in Reactors 4, 10, 11, and 17, the produced biogas was less than 100 mL/g vs due to the high amount of inoculum (60 %). The high amount of inoculum in the mixture causes insufficient supply of nutrients to the microorganisms reducing the biogas yield. Based on the findings of Dareioti and Kornaros [18], cheese whey has low levels of nitrogen, which increases the C/N proportion in this matter. The study by Cuetos, Gomez, Martinez, Fierro and Otero [19] also shows that considering the high nitrogen content in the blood, this matter had a high C/N proportion and could lead to system stability and optimized C/N proportion of the mixture. On this basis, Reactors 2 and 3 had 35 %-55 % blood and the amount of cheese whey was less than 40 %; this increased biogas production to more than 140 mL/g vs which was due to the pH stability of sheep blood and preventing acidification of the mixture. The highest performance of methane production was related to Reactors 10 and 17. These reactors were filled with a mixture of 60 % inoculum and 40 % cheese whey. The high amount of chesse whey caused the reactor to become unstable in terms of pH. As a result, they were ignored.

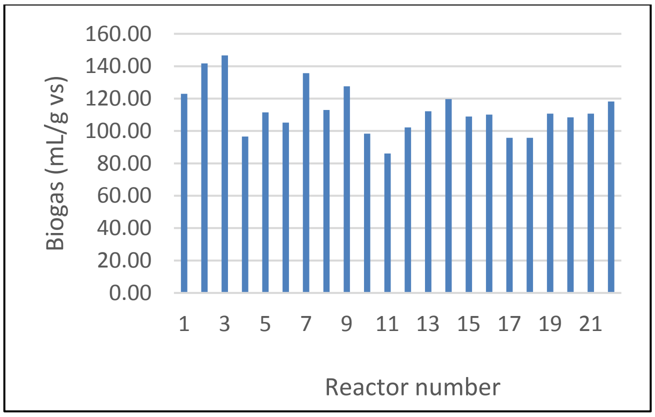

Figure 2. Cumulative chart related to biogas production

Whey presents a typical acidic pH value (5.4 ± 0.2) for these types of substrates, but is not suitable for the methanogenic step of AD. The organic fraction represented by VS/TS was 90.5 ± 4 %, indicating the high organic content of the substrate. C/N ratio was 15, indicating the high nitrogen content of the whey, but also the lactose content, which is an inherited characteristic of the substrate. It is worth mentioning that several authors have pointed out a higher cheese whey C/N ratio [20, 21].

The inoculum presented a pH of 7 ± 0.1, which is within the acceptable range reported in the literature for anaerobic digestion processes (6.6–7.9) [20, 22].

According to Vivekanand, Mulat, Eijsink, and Horn [21], biogas production potential of the three feedstocks fish ensilage, manure and whey was evaluated using Biochemical Methane Potential (BMP) tests. Since anaerobic digestion of single substrates may be inefficient due to imbalances in the carbon-nitrogen ratio, degree of biodegradability and/or due to lack of nutrients needed by the microbial community, co-digestion of these substrates was also assessed, revealing synergistic effects and a particularly good effect of combining manure with fish ensilage.

Cuetos et al. [23] showed that the addition of activated carbon for the digestion of residual blood significantly improved the digestion process. The adsorption capacity of ammonium, the protection this carrier may offer by limiting mass transfer of toxic compounds, and its toxic capacity as a conductive material may explain the successful digestion of residual blood as the sole substrate.

3.2. ANOVA analysis

3.2.1. Fit summary

This table includes significant statistics for choosing the right starting point for the final model. Based on these statistics, the appropriate model(s) is selected. According to the output related to amount of biogas, the software presented a special Quartic vs Quadratic model (see Table 3).

3.2.2. Coefficients in terms of coded factors

The calculation of the coefficient represents the predicted change in response per unit change in factor value if all the remaining variables are kept constant. The cut-off is the total average response of all the runs in an orthogonal design. Based on the factor settings, the coefficients are adaptations around that average. The VIFs are 1 when the variables are orthogonal; VIFs greater than 1 display multi-colinearity, the greater the VIF, the more extreme the association of factors. VIFs lower than 10 are tolerable as a rough norm (Table 4).

Table 3. Fit summary for the amount of biogas yield

Source	Sequential p-value	Lack of fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0354	0.0298	0.2225	0.1144	
Quadratic	0.0121	0.0578	0.5249	0.3847	
Special cubic	0.0871	0.0690	0.5858	0.4505	
Cubic	0.4918	0.0619	0.5732	0.0071	
Sp Quartic vs Quadratic	**0.0059**	**0.1470**	**0.7687**	**0.6061**	**Suggested**
Quartic vs Cubic	0.2909	0.0628	0.6515	-0.8846	
Quartic vs Sp Quartic	0.9335	0.0628	0.6515	-0.8846	

Table 4. Coefficients in terms of coded factors

Component	Coefficient estimate	df	Standard error	95 % CI Low	95 % CI High	VIF
A: blood	120.53	1	4.90	109.94	131.12	2.44
B: cheesse whey	107.44	1	4.91	96.84	118.04	2.56
C: inoculum	53.38	1	43.19	-39.92	146.67	60.57
AB	14.51	1	23.96	-37.25	66.27	2.96
AC	-10.43	1	79.65	-182.50	161.63	24.85
BC	74.74	1	82.96	-104.49	253.97	34.95
A^2BC	2481.59	1	599.77	1185.85	3777.32	5.48
AB^2C	524.61	1	610.47	-794.22	1843.45	5.68
ABC^2	-1545.33	1	677.32	-3008.60	-82.06	5.95

3.2.3. Final equation in terms of L-Pseudo components

In terms of coded variables, the equation can be used to make predictions about the answer to each factor's given levels. The high levels of the components of the mixture are coded by default as +1 and the low levels are coded as 0. By comparing the factor coefficients, the coded equation is helpful in identifying the relative influence of the factors.

$$Biogas = 120.53A + 107.44B + 53.38C + 14.51AB - 10.43AC + 74.74BC + 2481.59A^2BC + 524.61AB^2C - 1545.33ABC^2$$

3.3. Diagnostics plots

3.3.1. Normal plot

This plot demonstrates the distribution of residuals. If it follows a direct line, the residuals enjoy a good distribution and the model is desirable (see Figure 3).

3.3.2. Predicted vs actual plot

A graph of the response values is predicted against the actual response values. The objective is to find a value or a group of values that the model does not easily predict (see Figure 4).

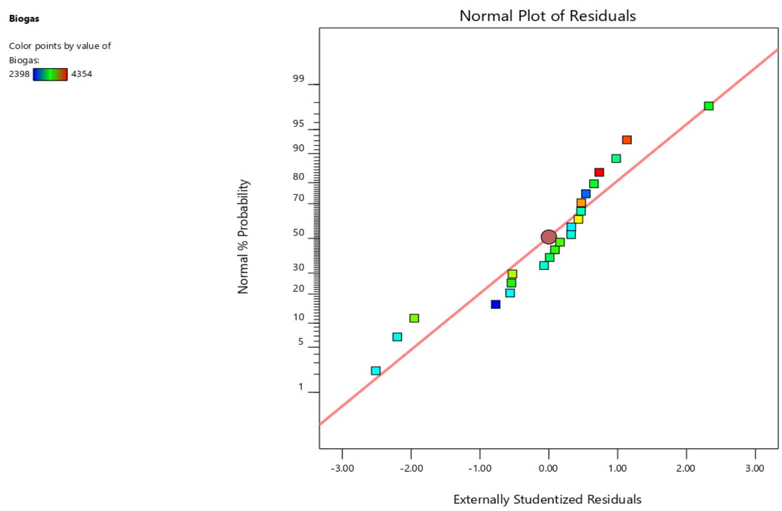

Figure 3. Standard plot of residuals for amount of biogas yield

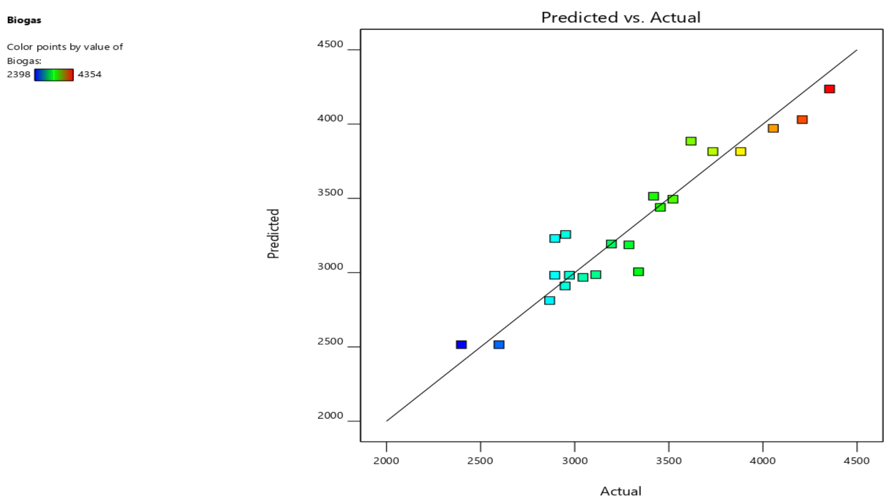

Figure 4. Predicted vs actual plots for amount of biogas yield

3.3.3. Box-Cox plot

Box-Cox plot helps identify the optimal power transfer function to be applied to the response. In the Box-Cox plot, the lowest point shows the amount of Lambda that contributes to the minimum residual number of squares in the transfer model. The current Lambda is within this range; according to Figure 5, there is no need for the transfer function to be added. The best Lambda value indicated by the plot, however, is -3.

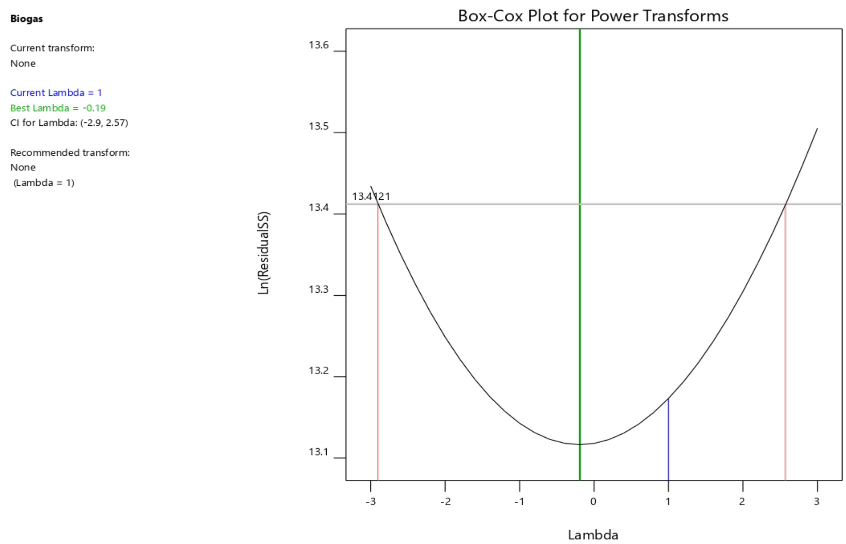

Figure 5. Box-Cox plot for biogas yield

3.4. Model graphs

3.4.1. Trace plot

For non-mixture designs, trace plots are identical to disturbance plots. They are used to compare the effects of all the components in the design space. To set the reference blend from which the traces are plotted, the factors tool is employed. The objective is to decide how sensitive the response near the reference blend is to deviation from the formulation. The reference blend is better defined by numerical optimization results, but defaults to the values of the centroid values (see Figure 6).

3.4.2. Contour plot and (3D) surface

This plot is a two-dimensional display of the response represented against the composition of the components of the mixture and illustrates the relationship between them. In this triangular graph, any of its vertices shows the maximum value for each matter, and as we move toward the side in front of each vertex, this value decreases. On the other hand, the color inside the triangle represents the response value. Accordingly, responses are determined from low to high using blue-red colors. The similar responses are linked together by a specified line. The responses that are in the red area of the graph demonstrate the optimal composition of the substrate matters, which are likely due to the optimal C/N proportion. Besides, low pH of cheese whey caused the red points in the graph to move toward compositions with more blood (see Figure 7). (3 D) Surface graph is the 3D contour plot with a slope and curve shape besides color (see Figure 8).

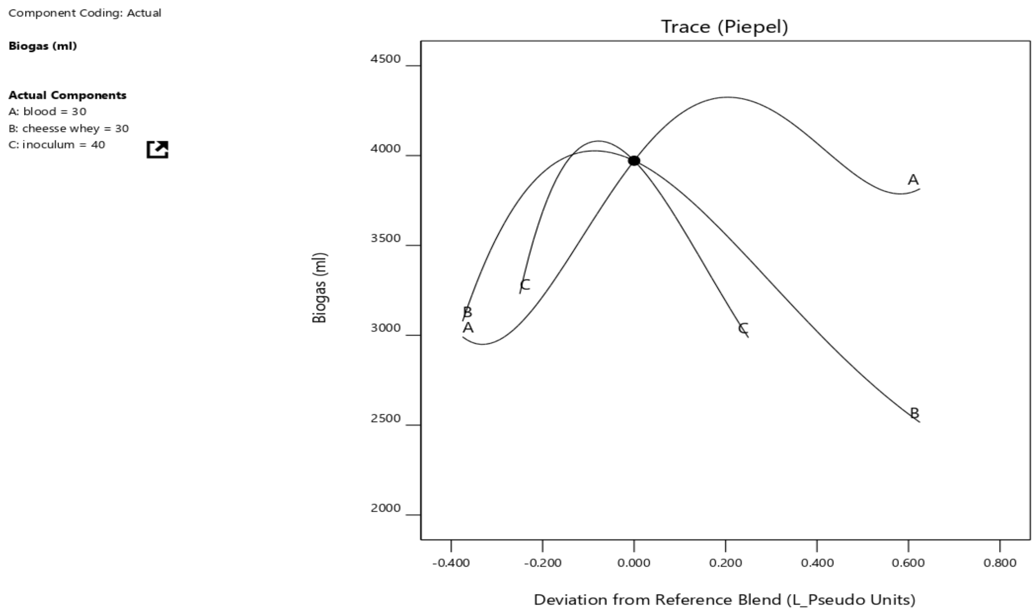

Figure 6. Trace plot for amount of biogas yield

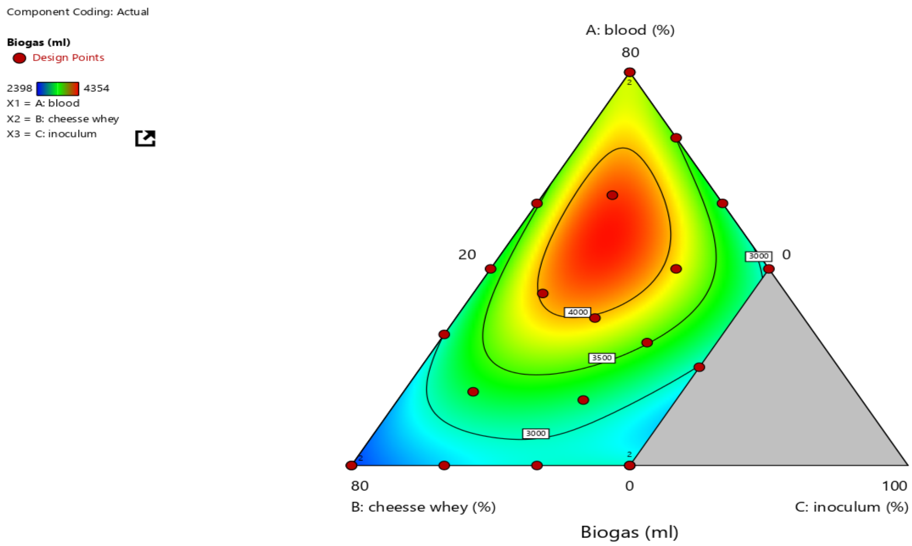

Figure 7. Contour plot for biogas yield

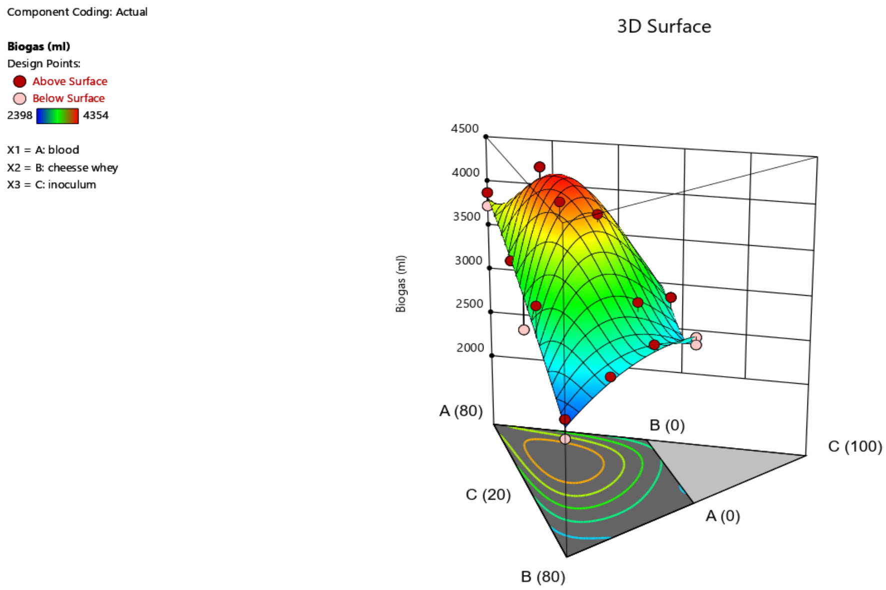

Figure 8. (3D) Surface graph for biogas yield

3.5. Optimization ramps

These ramps represent a graphical view of the optimal compositions. The optimal value for each independent variable are shown in red and those related to response (dependent variable) are in blue. According to the optimization, as shown in Figure 9, a composition consisting of 44 % sheep blood, 24 % cheese whey, and 32 % inoculum will produce the highest biogas possible, i.e., 143 mL/g vs of biogas.

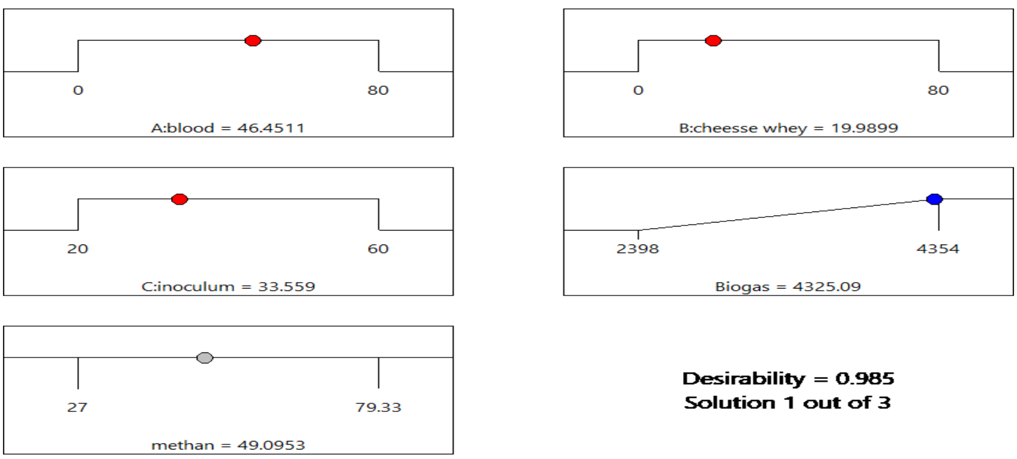

Figure 9. Optimization ramps for the highest biogas yield

4. CONCLUSIONS

Anaerobic co-digestion of three substances, including sheep blood, cheese whey, and inoculum, was investigated in the present study. The results can be summarized in the following. Design Expert software can be used as a practical program for designing experiments for the purpose of mixing different types of waste for biogas production. Using the mixture design enabled us to achieve optimal compositions of different matters for anaerobic co-digestion and measure these compositions together. In this study, the results related to anaerobic co-digestion of sheep blood, cheese whey, and inoculum showed that in the experimental phase, the optimal composition included 35 % sheep blood, 35 % cheese whey, and 30 % inoculum, which produced a biogas yield of 146.66 mL/g vs in a hydraulic retention time of 21 days. The obtained results were fed to the software for modeling and after modeling, the Design Expert software predicted an optimal composition that included 44 % sheep blood, 24 % cheese whey, and 32 % inoculum. The biogas yield in this prediction was 143 mL/g vs. Based on the findings, to overcome acidification in digestion of matters such as cheese whey, the composition of matters with higher constant pH can be used to increase the amount of the produced biogas and methane in a particular period. Furthermore, using inoculum accelerates the digestion operations due to abundant microorganisms and saves time and energy. Anaerobic digestion of wastes such as blood and cheese whey with a high COD can not only produce clear energies but also prevent environmental pollution to a considerable extent.

5. ACKNOWLEDGEMENT

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